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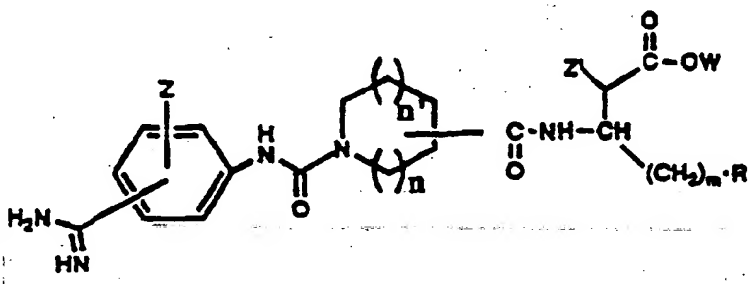

(I)

(57) Abstract

Novel urea derivatives of formula (I) are provided which inhibit platelet aggregation. This invention also pertains to pharmaceutical compositions and methods of using such derivatives. In formula (I) R, Z, Z', W, n, n' and m are as defined in th application.

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UREA DERIVATIVES USEFUL AS PLATELET AGGREGATION INHIBITORS

Background of the Invention

Field of the Invention

This invention pertains to urea derivatives which inhibit platelet aggregation in mammals.

Related Art

Fibrinogen is a glycoprotein present as a normal component of blood plasma. It participates in platelet aggregation and fibrin formation in the blood clotting mechanism.

Platelets are cellular elements found in whole blood which also participate in blood coagulation. Fibrinogen binding to platelets is important to normal platelet function in the blood coagulation mechanism. When a blood vessel receives an injury, the platelets binding to fibrinogen will initiate aggregation and form a thrombus. Interaction of fibrinogen with platelets occurs through a membrane glycoprotein complex, known as gpIIb/IIIa; this is an important feature of the platelet function. Inhibitors of this interaction are useful in modulating or preventing platelet thrombus formation.

It is also known that another large glycoprotein named fibronectin, which is a major extracellular matrix protein, interacts with fibrinogen and fibrin, and with other structural molecules such as actin, collagen and proteoglycans. Various relatively large polypeptide fragments in the cell-binding domain of fibronectin have been found to have cell-attachment activity. (See U.S. Patents 4,517,686, 4,589,881, and 4,661,111). Certain relatively short peptide fragments from the same m lecule were found to pr mot cell attachment

to a substrate when immobilized on the substrate or to inhibit attachment when in a solubilized or suspended form. (See U.S. Patents 4,578,079 and 4,614,517).

In U.S. Patent 4,683,291, inhibition of platelet function is disclosed with synthetic peptides designed to be high affinity antagonists of fibrinogen binding to platelets. U.S. Patent 4,857,508 discloses tetrapeptides having utility as inhibitors of platelet aggregation.

Other synthetic peptides and their use as inhibitors of fibrinogen binding to platelets are disclosed by Koczewiak et al., Biochem. 23, 1767-1774 (1984); Plow et al., Proc. Natl. Acad. Sci. 82, 8057-8061 (1985); Ruggeri et al., Ibid. 83, 5708-5712 (1986); Ginsberg et al., J. Biol. Chem. 260 (7), 3931-3936 (1985); Haverstick et al., Blood 66 (4), 946-952 (1985); and Ruoslahti and Pierschbacher, Science 238, 491-497 (1987). Still other such inhibitory peptides are disclosed in EP Patent Applications 275,748 and 298,820.

U.S. Patent 4,879,313 discloses guanidino substituted alkanoic acid derivatives which inhibit protein to receptor binding and are useful for the treatment of thrombosis and cardiac infarction.

European Patent Application 372,486 discloses N-acyl beta amino acid derivatives and their salts. Said compounds are useful for inhibiting platelet aggregation in the treatment of thrombosis, stroke, myocardial infarction, inflammation and arteriosclerosis, and for inhibiting metastasis.

European Patent Application 381,033 discl ses amidino or guanidinoaryl substituted alkanoic acid derivatives useful for the treatment

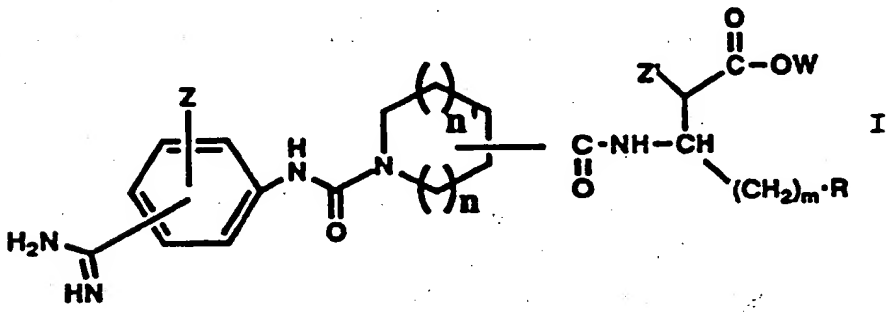
of thrombosis, apoplexy, cardiac infarction, inflammation, arteriosclerosis and tumors.

European Patent Application 445,796 discloses Acetic Acid derivatives useful as a ligand for adhesive proteins on platelets. As such these compounds are useful to modulate and/or inhibit platelet aggregation.

European Patent Application 513,810 discloses amidinoaryl substituted alkanoic acid derivatives which have usefulness as inhibitors of platelet aggregation, the disclosure of which is hereby incorporated by reference.

Summary of the Invention

The present invention relates to a class of compounds represented by the formula:



I

or a pharmaceutically acceptable salt thereof,

wherein R is independently selected from the group consisting of hydrogen, lower alkyl radicals, lower alkenyl radicals, lower alkynyl radicals, alicyclic hydrocarbon radicals, and aromatic hydrocarbon radicals, wherein all of said radicals are optionally substituted with hydroxyl, lower alkoxy, lower alkyl, halogen, nitro, carboxyl, trifluoromethyl, amino, acyloxy, phenyl and naphthyl which are optionally substituted with halogen, nitro, lower alkoxy, and lower alkyl; or R is a monocyclic or bicyclic heterocyclyl radical in which

1 to about 3 h teroatoms are independently sel cted from nitrogen, oxygen or sulfur and said heterocyclyl radicals may be optionally substituted with one or more groups selected from alkyl having 1 to 6 carbon atoms, alkoxy having 1 to 6 carbon atoms, halo, and hydroxy;

W is selected from the group consisting of hydrogen, lower alkyl radicals, lower alkenyl radicals, lower alkynyl radicals, alicyclic hydrocarbon radicals and aromatic hydrocarbon radicals, wherein all of said radicals are optionally substituted with hydroxyl, lower alkyl, lower alkoxy, halogen, nitro, amino, acyloxy, phenyl and naphthyl which may be optionally substituted with halogen, nitro, lower alkoxy, and lower alkyl;

Z, Z' are independently selected from the group consisting of hydrogen, lower alkyl radicals, halogen, alkoxy, cyano, sulfonyl, and hydroxy radicals;

m is an integer from 0 to about 6;

n is an integer from 0 to about 3;

n' is an integer from 0 to about 3.

R is preferably a mono or bi cyclic heterocyclyl radical wherein 1 to 2 hetero atoms are independently selected from nitrogen and oxygen. More preferably R is a monocyclic heterocyclyl radical wherein 1 to 2 hetero atoms are nitrogen. Most preferably R is pyridine.

W is preferably a lower alkyl and hydrogen.

Z,Z' is preferably hydrogen and lower alkyl, more preferably hydrogen.

m is preferably 0 to 3, more preferably 0.

n is preferably 0 to 2, more preferably 0 to 1.

n' is preferably 0 to 2, more preferably 1 to 2.

The invention further relat s to pharmaceutical compositions comprising a compound of Formula (I). Such compounds and compositions have usefulness as inhibitors of platelet aggregation.

It is still another object of the invention to provide a method to therapeutically inhibit or modulate platelet aggregation or the like in a mammal in need of such treatment with a compound of the formula I in unit dosage form. Particularly in inhibiting or modulating platelet aggregation by administering an amount between 0.5mg/kg to 10mg/kg, preferably 3mg/kg to an animal in need thereof.

Many other objects and purposes of the invention will be clear from the following detailed description of the invention and examples.

Detailed Description of the Invention

A preferred embodiment of the present invention is a compound of formula I or a pharmaceutically acceptable salt thereof;

wherein R is independently selected from the group consisting of hydrogen, lower alkyl radicals of 1 to about 6 carbon atoms, lower alkenyl radicals of 2 to about 6 carbon atoms, lower alkynyl radicals of 2 to about 8 carbon atoms, alicyclic hydrocarbon radicals of 3 to about 6 carbon atoms, and aromatic hydrocarbon radicals, wherein all of said radicals are optionally substituted with hydroxyl, lower alkoxy, lower alkyl, halogen, nitro, carboxyl, and trifluoromethyl; or R is a monocyclic or bicyclic heterocyclyl radical in which 1 to about 3 heteroatoms are independently selected from nitrogen or oxygen;

W is selected from the group consisting of hydrogen, l wer alkyl radicals of 1 to about 6 carbon atoms, lower alkenyl radicals of 2 to about 6 carbon atoms, alicyclic hydrocarbon radicals of 3 to

about 6 carbon atoms, and aromatic hydrocarbon radicals of 6 to about 12 carbon atoms, wherein all of said radicals are optionally substituted with hydroxyl, lower alkoxy, lower alkyl, halogen, nitro, amino, and acyloxy;

Z, Z' are independently selected from the group consisting of hydrogen, lower alkyl radicals, halogen, alkoxy, cyano, sulfonyl, and hydroxy radicals;

m is an integer from 0 to about 6;

n is an integer from 0 to about 3;

n' is an integer from 0 to about 3.

Another preferred embodiment of the present invention is a compound of formula I or a pharmaceutically acceptable salt thereof:

wherein R is independently selected from the group consisting of hydrogen, lower alkyl radicals and aromatic hydrocarbon radicals, or R is a monocyclic heterocyclyl radical in which 1 to about 2 hetero atoms are nitrogen;

W is selected from the group consisting of hydrogen and lower alkyl radicals;

Z, Z' are independently selected from the group consisting of hydrogen, lower alkyl radicals, halogen, and hydroxy radicals;

m is an integer from 0 to about 3;

n is an integer from 0 to about 3;

n' is an integer from 0 to about 3.

As utilized herein, the term "lower alkyl", alone or in combination, means an acyclic alkyl radical containing from 1 to about 10, preferably from 1 to about 8 carbon atoms and more preferably 1 to about 6 carbon atoms. Examples of such radicals include methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, sec-butyl, tert-butyl, pentyl, isoamyl, hexyl, octyl and the like.

The term "lower alkenyl" r fers to an unsaturated acyclic hydrocarbon radical in so much as it contains at least one double bond. Such radicals containing from about 2 to about 10 carbon atoms, preferably from about 2 to about 8 carbon atoms and more preferably 2 to about 6 carbon atoms. Examples of suitable alkenyl radicals include propylenyl, buten-1-yl, isobutenyl, penten-1-yl, 2-2-methylbuten-1-yl, 3-methylbuten-1-yl, hexen-1-yl, hepten-1-yl, and octen-1-yl, and the like.

The term "lower alkynyl" refers to an unsaturated acyclic hydrocarbon radicals in so much as it contains one or more triple bonds, such radicals containing about 2 to about 10 carbon atoms, preferably having from about 2 to about 8 carbon atoms and more preferably having 2 to about 6 carbon atoms. Examples of suitable alkynyl radicals include ethynyl, propynyl, butyn-1-yl, butyn-2-yl, pentyn-1-yl, pentyn-2-yl, 3-methylbutyn-1-yl, hexyn-1-yl, hexyn-2-yl, hexyn-3-yl, 3,3-dimethylbutyn-1-yl radicals and the like.

The term "alicyclic hydrocarbon" means a aliphatic radical in a ring with 3 to about 10 carbon atoms, and preferably from 3 to about 6 carbon atoms. Examples of suitable alicyclic radicals include cyclopropyl, cyclopropylenyl, cyclobutyl, cyclopentyl, cyclohexyl, 2-cyclohexen-1-ylenyl, cyclohexenyl and the like.

The term "aromatic hydrocarbon radical" means 4 to about 16 carbon atoms, preferably 6 to about 12 carbon atoms, more preferably 6 to about 10 carbon atoms. Examples of suitable aromatic hydrocarbon radicals include phenyl, naphthyl, and the lik .

The term "lower alkoxy", alon or in combination, means an alkyl ether radical wher in

the term alkyl is as defined above and most preferably containing 1 to about 4 carbon atoms. Examples of suitable alkyl ether radicals include methoxy, ethoxy, n-propoxy, isopropoxy, n-butoxy, iso-butoxy, sec-butoxy, tert-butoxy and the like.

The term "heterocyclyl radical" means a heterocyclyl hydrocarbon radical preferably an aromatic heterocyclyl hydrocarbon radical with 4 to about 10 carbon atoms, preferably about 5 to about 6; wherein 1 to about 3 carbon atoms are replaced by nitrogen, oxygen or sulfur. The "heterocyclyl radical" may be fused to a aromatic hydrocarbon radical or to another heterocyclyl radical. The "heterocyclyl radical" may be saturated, partially saturated, or fully unsaturated. Suitable examples include pyrrolyl, pyridinyl, pyrazolyl, triazolyl, pyrimidinyl, pyridazinyl, oxazolyl, thiazolyl, imidazolyl, indolyl, thiophenyl, furanyl, tetrazolyl, 2-pyrrolinyl, 3-pyrrolinyl, pyrrolidinyl, 1,3-dioxolanyl, 2-imidazolinyl, imidazolidinyl, 2-pyrazolinyl, pyrazolidinyl, isoxazolyn, isothiazolyn, 1,2,3-oxadiazolyn, 1,2,3-triazolyn, 1,3,4-thiadiazolyn, 2H-pyranyl, 4H-pyranyl, piperidinyl, 1,4-dioxanyl, morpholinyl, 1,4-dithianyl, thiomorpholinyl, pyrazinyl, piperazinyl, 1,3,5-triazinyl, 1,3,5-trithianyl, benzo(b)thiophenyl, benzimidazolyn, quinolinyl, and the like.

The term halogen means fluorine, chlorine, bromine or iodine.

The term "pharmaceutically acceptable salt" refers to a salt prepared by contacting a compound of formula (I) with an acid whose anion is generally consid red suitable for human consumption. Examples of pharmacologically acceptable salts include the hydrochloride, hydrobromide, hydr i dide, sulfate,

phosphate, acetate, propionate, lactate, maleate, oxalate, malate, succinate, tartrate and citrate salts. All of these salts may be prepared by conventional means by reacting, for example, the appropriate acid with the corresponding compound of formula I.

Suitable pharmaceutically-acceptable base addition salts of compounds of Formula I include metallic salts made from aluminum, calcium, lithium, magnesium, potassium, sodium and zinc or organic salts made from N,N'-dibenzylethylenediamine, chloroprocaine, choline, diethanolamine, ethylenediamine, meglumine (N-methylglucamine) and procaine.

Total daily dose administered to a host in need in single or divided doses may be in amounts, for example, from 0.001 to 100 mg/kg body weight daily and more usually 0.01 to 10 mg. Dosage unit compositions may contain such amounts of submultiples thereof to make up the daily dose.

The amount of active ingredient that may be combined with the carrier materials to produce a single dosage form will vary depending upon the host treated and the particular mode of administration.

It will be understood that the specific dose level for any particular patient will depend upon a variety of factors including the activity of the specific compound employed, the age, body weight, general health, sex, diets, time of administration, route of administration, rate of excretion, drug combination, and the severity of the particular disease undergoing therapy.

The compounds of the present invention may be administered orally, parenterally, by inhalation spray, rectally, or topically in dosage unit formulations containing conventional nont xic

pharmaceutically acceptable carriers, adjuvants, and vehicles as desired. Injectable preparations, for example, sterile injectable aqueous or oleaginous suspensions may be formulated according to the known art using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation may also be a sterile injectable solution or suspension in a nontoxic parenterally acceptable diluent or solvent, for example, as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution, and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil may be employed including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid find use in the preparation of injectable.

Suppositories for rectal administration of the drug can be prepared by mixing the drug with a suitable nonirritating excipient such as cocoa butter and polyethylene glycols which are solid at ordinary temperature but liquid at the rectal temperature and will therefore melt in the rectum and release the drug.

Solid dosage forms for oral administration may include capsules, tablets, pills, powders, and granules. In such solid dosage forms, the active compound may be admixed with at least one inert diluent such as sucrose, lactose or starch. Such dosage forms may also comprise, as in normal practice, additional substances other than inert dilu nts, .g., lubricating agents such as magnesium stearate. In the case f capsules, tablets, and pills, the dosage forms may also comprise buffering

agents. Tablets and pills can additionally be prepared with enteric coatings.

Liquid dosage forms for oral administration may include pharmaceutically acceptable emulsions, solutions, suspensions, syrups, and elixirs containing inert diluents commonly used in the art, such as water. Such compositions may also comprise adjuvants, such as wetting agents, emulsifying and suspending agents, and sweetening, flavoring, and perfuming agents.

While the compounds of the invention can be administered as the sole active pharmaceutical agent, they can also be used in combination with one or more active pharmaceutical agents. When administered as a combination, the therapeutic agents can be formulated as separate compositions which are given at the same time or different times, or the therapeutic agents can be given as a single composition.

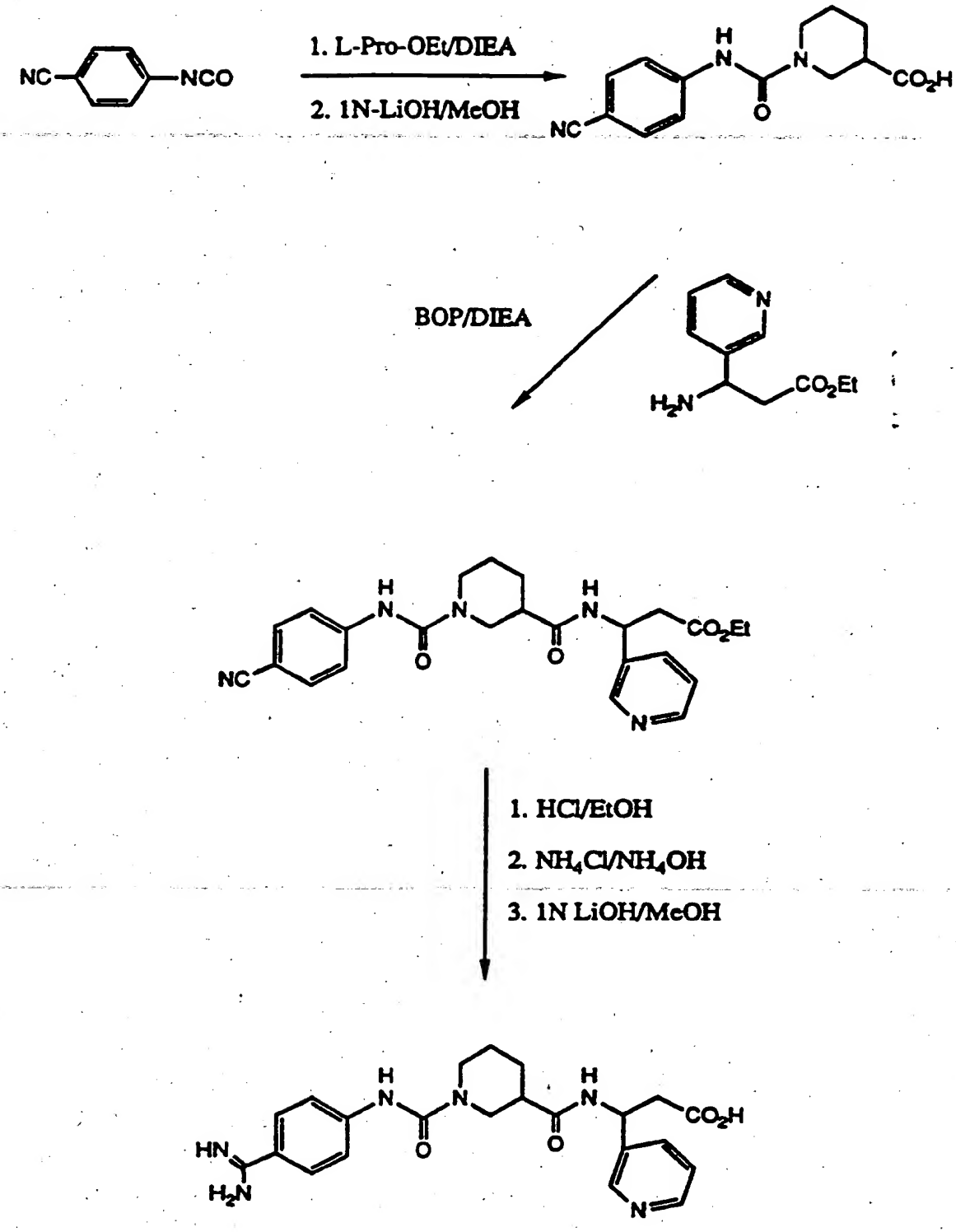
In the structures and formulas herein, the bond drawn across a bond of an aromatic ring can be to any available atom on the aromatic ring.

The compounds of formula I can exist in various isomeric forms and all such isomeric forms are meant to be included. Tautomeric forms are also included in the invention. Pharmaceutically acceptable salts of such isomers and tautomers are meant to be included as well.

The compounds listed above may be prepared by standard synthetic methods combined with methods analogous to solution phase peptide synthesis [see: The Peptides: Analysis, Synthesis, Biology (E. Gross and J. Meienhofer, eds.), (Vol. 1-5, Academic Press, New York)], the disclosure of which is hereby incorporated by reference.

General synthetic sequences for preparing the compounds of formula I are outlined in Scheme I.

SCHEME I


NC
NCO
1. L-Pro-OEt/DIEA
2. 1N-LiOH/MeOH
H
N
N
CO2H
O
NC
BOP/DIEA
N
H2N
CO2Et
H
N
N
H
N
CO2Et
O
O
NC
N
1. HCl/EtOH
2. NH4Cl/NH4OH
3. 1N LiOH/MeOH
HN
H2N
CO2H

Purification of final compounds can be performed by reverse phase high pressure liquid chromatography [High Performance Liquid Chromatography Protein and Peptide Chemistry, F. Lottspeich, A. Henscher, K.P. Hupe, eds.) Walter DeGruyter, New York, 1981, the disclosure of which is hereby incorporated by reference.) or crystallization.

Contemplated equivalents of the general formulas set forth above for the platelet aggregation inhibitors and derivatives as well as the intermediates are compounds otherwise corresponding thereto and having the same general properties wherein one or more of the various R groups are simple variations of the substituents as defined therein, e.g., wherein R is a higher alkyl group than that indicated. In addition, where a substituent is designated as, or can be, a hydrogen, the exact chemical nature of a substituent which is other than hydrogen at that position, e.g., a hydrocarbyl radical or a halogen, hydroxy, amino and the like functional group, is not critical so long as it does not adversely affect the overall activity and/or synthesis procedure.

The chemical reactions described above are generally disclosed in terms of their broadest application to the preparation of the compounds of this invention. Occasionally, the reactions may not be applicable as described to each compound included within the disclosed scope. The compounds for which this occurs will be readily recognized by those skilled in the art. In all such cases, either the reactions can be successfully performed by conventional modificati ns known to those skilled in th art, e.g., by appropriate protection f interfering groups, by changing to alternative

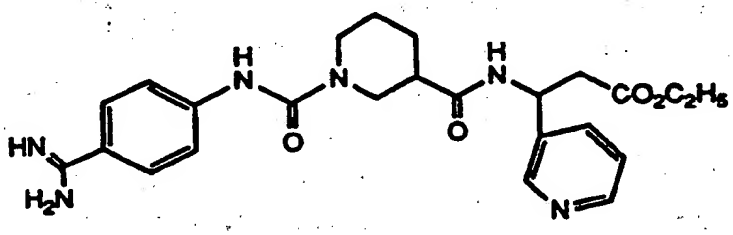
conventional reag nts, by routine modification of reaction conditions, and the like, or other reactions disclosed herein or otherwise conventional, will be applicable to the preparation of the corresponding compounds of this invention. In all preparative methods, all starting materials are known or readily preparable from known starting materials.

Without further elaboration, it is believed that one skilled in the art can, using the preceding description, utilize the present invention to its fullest extent. Therefore the following preferred specific embodiments are to be construed as merely illustrative and not limitative of the remainder of the disclosure in any way whatsoever.

The following examples are provided to illustrate the present invention and are not intended to limit the scope thereof. Those skilled in the art will readily understand that known variations of the conditions and processes of the following preparative procedures can be used to prepare these compounds. All temperatures expressed are in degrees centigrade.

Example 1

Ethyl β-[[N-3-[[[4-(aminoiminomethyl)phenyl]amino]carbonyl]piperidylcar-bonyl]amino]-3-pyridinepropanoate



Step 1. Preparation of N-3-[[(4-cyanophenyl)amino]carbonyl]piperidylcarboxylic acid

4-cyanophenylisocyanate (0.43 g; 3 mmoles), nipecotic acid ethyl ester.HCl (0.57 g; 3 mmoles) and N, N'- diisopropylethylamine (0.39g; 3 mmoles) were stirred in N,N-dimethylformamide (25 ml) overnight. The resulting ester was hydrolized by addition of 1 N LiOH (25 ml) and the acid was isolated by HPLC using acetonitrile/water/trifluoroacetic acid system. (FAB-MS : MH^+ = 274.1).

Step 2. Preparation of ethyl β-[[N-3-[[[4-(aminoiminomethyl)phenyl]amino]-carbonyl]piperidylcarbonyl]amino]-3-pyridinepropanoate

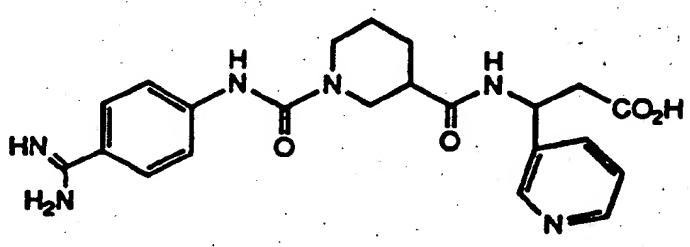
A portion of the acid (0.54 g; 2 mmoles) and ethyl β-[3-amino-(3-pyridyl)]-propanoate dihydrochloride (0.9g; 3.3 mmoles) were dissolved and stirred in N,N-dimethylformamide (25 ml). To this mixture, benzotriazol-1-yl-oxy-tris(dimethylamino)phosphonium hexafluorophosphate (0.88 g; 2 mmoles) and N,N-diisopropylethylamine (0.52 g; 4 mmoles) were added. The reaction was carried out for another hour and the mixture was then taken down to dryness. The residue was dissolved in ethanol (100ml) and the solution was saturated with HCl gas for 2 hrs. The reaction mixture was evaporated to dryness under reduced pressure and the remaining residue was redissolved in ethanol (100 ml). The solution was treated with ammonium chloride (1 g) in ammonium hydroxide (10 ml) at room temperature for 4hrs. Ethanol was removed on rotaevaporator and the residue was purified by HPLC using acetonitrile/water/trifluoroacetic acid. The desired fractions w re

collected and lyophilized to give 330 mg of white solid (37% yield). FAB-MS: MH^+ = 467.3.
Elemental analysis: $C_{24}H_{30}N_6O_4 \cdot 2CF_3COOH \cdot H_2O$

	C	H	N
Calculated:	47.19	4.77	11.79
Found:	46.47	4.39	11.63

Example 2

β-[[N-3-[[[4-(aminoiminomethyl)phenyl]amino]carbonyl]piperidylcar-bonyl]amino]-3-pyridinepropanoic acid

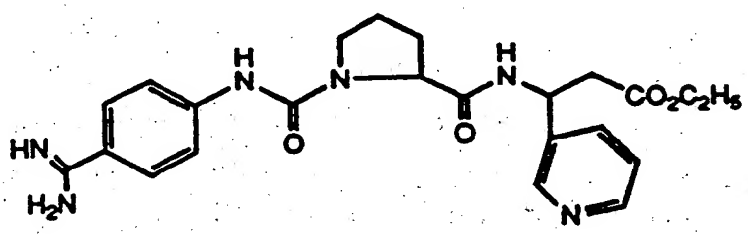


Ethyl β-[[N-3-[[[4-(aminoiminomethyl)-phenyl]amino]carbonyl]piperidylcar-bonyl]amino]-3-pyridinepropanoate.2 CF_3COOH (120 mg) was treated with 1N lithium hydroxide/methanol (1:1; 20 ml) for 5 min. Methanol was removed under reduced pressure and the pH was adjusted to 5 with 50% acetic acid. The crude product was purified by HPLC using acetonitrile/water/ trifluoroacetic acid solvent system. The desired fractions were collected and lyophilized to give 97 mg of white solid (89% yield). FAB-MS: MH^+ = 439.3.
Elemental analysis: $C_{22}H_{26}N_6O_4 \cdot 2CF_3COOH \cdot H_2O$

	C	H	N
Calculated:	45.61	4.38	12.28
Found:	45.05	3.98	12.24

Example 3

Ethyl β-[[N-2-[[[4-(aminoiminomethyl)phenyl]amino]carbonyl]pyrrolidylcar-bonyl]amino]-3-pyridinepropanoate



Step 1. Preparation of N-2-[[(4-cyanophenyl)amino]carbonyl]pyrrolidylcarboxylic acid

4-cyanophenylisocyanate (1.4 g; 10 mmoles), proline ethyl ester.HCl (1.5 g;8.3 mmoles) and N,N'-diisopropylethylamine (1.3g; 10 mmoles) were stirred in N,N'-dimethylformamide (50 ml) at room temperature for 4 hrs. The resulting ester was hydrolized by addition of 1 N LiOH (15 ml) and the acid was isolated by HPLC using acetonitrile/water/trifluoroacetic acid system. (FAB-MS : MH^+ = 260).

Step 2. Preparation of ethyl β-[[N-2-[[[4-(aminoiminomethyl)phenyl]amino]carbonyl]pyrrolidylcar-bonyl]amino]-3-pyridinepropanoate

A portion of the acid (0.37 g; 1.4 mmoles) and ethyl β-[3-amino(3-pyridyl)]-propanoate dihydrochloride (0.46 g; 1.5 mmoles) were dissolved and stirred in N,N-dimethylformamide (50 ml). To this mixture, benzotriazol-1-yl-oxy-tris(dimethylamino)phosphonium hexafluorophosphate (0.66 g; 1.5 mmoles) and N,N-diis propylethylamine (0.36 g; 3 mmoles) wer added. The reaction was carried out for another h ur and the mixture was th n taken down to dryn ss. The residu was

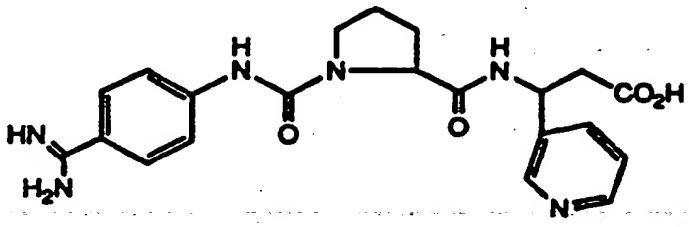
dissolved in ethanol (100ml) and the solution was saturated with HCl gas for 2 hrs. The reaction mixture was evaporated to dryness under reduced pressure and the remaining residue was redissolved in ethanol (100 ml). The solution was treated with ammonium chloride (1 g) in ammonium hydroxide (10 ml) at room temperature for 4hrs. Ethanol was removed on rotaevaporator and the residue was purified by HPLC using acetonitrile/water/ trifluoroacetic acid. The desired fractions were collected and lyophilized to give 490 mg of white solid (72% yield). FAB-MS: MH^+ = 453.2.

Elemental analysis: $C_{23}H_{28}N_6O_4 \cdot 2CF_3COOH \cdot H_2O$

	C	H	N
Calculated:	46.41	4.58	12.03
Found:	46.03	4.27	11.87

Example 4

β-[[N-2-[[[4-(aminoiminomethyl)phenyl]amino]carbonyl]pyrrolidylcar-bonyl]amino]-3-pyridinepropanoic acid



Step 2. Preparation of ethyl β-[[N-2-[[[4-(aminoiminomethyl)phenyl]amino]carbonyl]pyrrolidylcar-bonyl]amino]-3-pyridinepropanoate.2 CF_3COOH (280 mg) was treated with 1N lithium hydroxide/methanol (1:1; 20 ml) for 5 min. Methanol was removed under reduced pressur and the pH was adjusted to 5 with 50% acetic acid. The crud product was purified by HPLC using acetonitrile/ wat r/trifluoroac tic acid

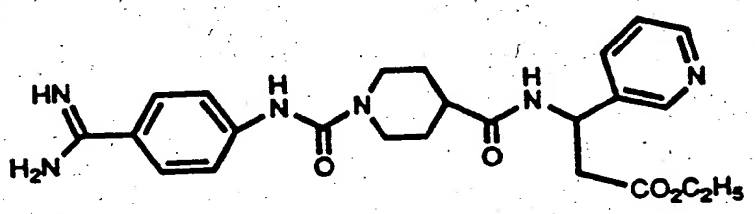
solvent system. The desired fractions were collected and lyophilized to give 241 mg of white solid (92% yield). FAB-MS: MH^+ = 425.1.

Elemental analysis: $C_{21}H_{24}N_6O_4 \cdot 2CF_3COOH \cdot H_2O$

	C	H	N
Calculated:	44.77	4.17	12.53
Found:	44.96	3.94	12.17

Example 5

Ethyl β-[[N-[[[4-(aminoiminomethyl)phenyl]amino]carbonyl]4-piperidylcarbonyl]-amino]-3-pyridinepropanoate



Step 1. Preparation of N-[[(4-cyanophenyl)amino]carbonyl]4-piperidylcarboxylic acid

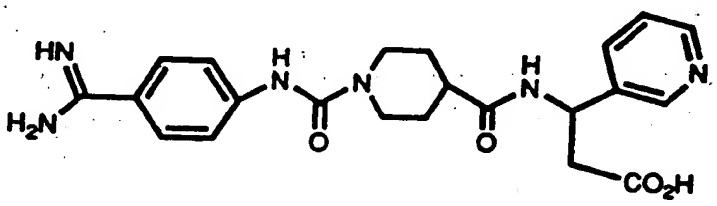
4-cyanophenylisocyanate (0.72 g; 5 mmoles), isonipecotic acid (0.65 g; 5 mmoles) and 1N sodium hydroxide (5 ml) were stirred in N,N-dimethyl-formamide (25 ml) overnight. The acid was isolated by HPLC using acetonitrile/water/trifluoroacetic acid system. (FAB-MS : MH^+ = 274.1).

Step 2. Preparation of ethyl β-[[N-[[[4-(aminoiminomethyl)phenyl]amino]-carbonyl]4-piperidylcarbonyl]amino]-3-pyridinepropanoate

A portion f the acid (0.14 g; 0.5 mmoles) and ethyl β-[amino-(3-pyridyl)]-propanoate dihydrochloride (0.14 g; 0.5 mmoles) were dissolved and stirred in N,N-dimethylformamide (25 ml). To this mixture, benzotriazol-1-yl-oxy-tris(dimethylamino)phosphonium hexafluoro-phosphate (0.22 g; 0.5 mmoles) and N,N-diisopropylethylamine (0.07 g; 0.5 mmoles) were added. The reaction was carried out for another hour and the mixture was then taken down to dryness. The residue was dissolved in ethanol (20ml) and the solution was saturated with HCl gas for 2 hrs. The reaction mixture was evaporated to dryness under reduced pressure and the remaining residue was redissolved in ethanol (20 ml). The solution was treated with ammonium chloride (0.2 g) in ammonium hydroxide (2 ml) at room temperature for 4hrs. Ethanol was removed on rotaevaporator and the residue was purified by HPLC using acetonitrile/water/ trifluoroacetic acid. The desired fractions were collected and lyophilized to give 160 mg of white solid. FAB-MS: MH^+ = 467.3.

Example 6

β-[[N-[[[4-(aminoiminomethyl)phenyl]amino]carbonyl]4-piperidylcarbonyl]amino]-3-pyridinepropanoic acid



Ethyl β-[[N-[[[4-(aminoiminomethyl)phenyl]amino]carbonyl]4-piperidylcarbonyl]-amino]-3-pyridinepropanoate(160 mg) was treated with 1N lithium hydroxide/methanol (1:1; 10 ml) for 10 min. Methanol was removed under reduced pressure and the pH was adjusted to 5 with 50% acetic acid. The crude product was purified by HPLC using acetonitrile/water/trifluoroacetic acid solvent system.The desired fractions were collected and lyophilized to give 135 mg of white solid (62% yield). FAB-MS: MH^+ = 439.4.

Elemental analysis: $C_{22}H_{26}N_6O_4 \cdot 2CF_3COOH \cdot H_2O$

	C	H	N
Calculated:	45.61	4.38	12.28
Found:	45.97	4.18	12.33

In-Vitro Platelet Aggregation in PRP

Healthy male or female dogs were fasted for 8 hours prior to drawing blood; then 30 ml whole blood was collected using a butterfly needle and 30 cc plastic syringe with 3 ml of 0.129 M buffered sodium citrate (3.8%). The syringe was rotated carefully as blood was drawn to mix the citrate. Platelet-rich plasma (PRP) was prepared by centrifugation at 975 x g for 3.17 minutes at room temperature allowing the centrifuge to coast to a stop without braking. The PRP was removed from the blood with a plastic pipette and placed in a plastic capped, 50 mL Corning conical sterile centrifuge tube which was held at room temperature. Platelet poor plasma (PPP) was prepared by centrifuging the remaining blood at 2000 x g for 15 minutes at room temperature allowing the centrifuge to coast to a stop without braking. The PRP was adjusted with PPP to a count of 2-3 x 10^8 platelets per mL. 400 uL of the PRP preparation and 50 uL of the compounds solution to be tested or saline were pre incubated

for 1 minute at 37°C in a BioData, Horsham, PA). 50 uL of adenosine 5' diphosphate (ADP) (50 um final concentration) was added to the cuvettes and the aggregation was monitored for 1 minute. All compounds are tested in duplicate. Results are calculated as follows: Percent of control = [(maximal OD minus initial OD of compound) divided by (maximal OD minus initial OD of control saline)] x 100. The % inhibition = 100 - (percent of control).

The compounds tested and their median inhibitory concentrations (IC_{50}) are recorded in Table I. IC_{50}'s (dosage at which 50% of platelet aggregation is inhibited) were calculated by linear regression of the dose response curve. The assay results for the compounds of Examples 1 to 6 are set forth in Table I below.

INHIBITION OF EX VIVO COLLAGEN INDUCED AGGREGATION BY COMPOUNDS OF THE INVENTION

The purpose of this assay is to determine the effects of antiplatelet compounds on ex vivo collagen induced platelet aggregation when administered either intravenously or orally to dogs.

Pretreatment (control) blood samples are drawn from either conscious or anesthetized dogs (Beagles) and centrifuged to prepare platelet rich plasma (PRP). Aggregatory response to collagen is measured in a aggregometer and used as Control. Compounds are administered, either intragasterically (either by capsule or stomach tube or intravenously). Blood samples are drawn at predetermined intervals after compound administration, PRP prepared and aggregation to collagen determin d. Compound inhibition of aggregation is determined by c mparing th

aggregation r sponse after compound administration to the pretreatment response.

Table I

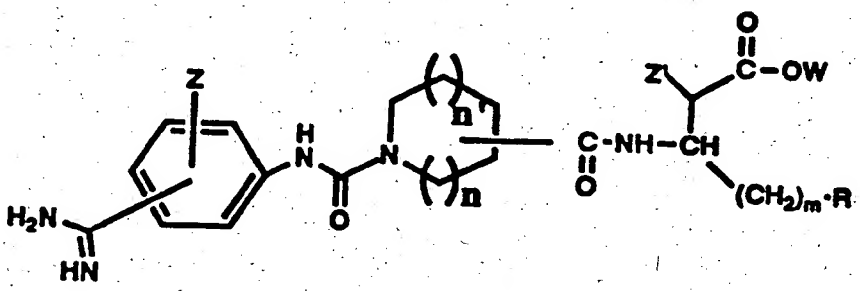
Example	Dog PRP IC_{50} or % Inh.	Ex Vivo Effect after IG Administration
1	NT	NT
2	8.5 X 10^{-7}M	*
3	NT	NT
4	30% at 10^{-5}M	NT
5	NT	NT
6	19% at 10^{-6}M	NT

NT - not tested
* - not active at the dose tested

From the foregoing description, one skilled in the art can easily ascertain the essential characteristics of this invention, and without departing from the spirit and scope thereof, can make various changes and modifications of the invention to adapt it to various usages and conditions.

WHAT IS CLAIMED IS:

1. A compound or a pharmaceutically acceptable salt, thereof having the formula:



wherein R is independently selected from the group consisting of hydrogen, lower alkyl radicals, lower alkenyl radicals, lower alkynyl radicals, alicyclic hydrocarbon radicals, and aromatic hydrocarbon radicals, wherein all of said radicals are optionally substituted with hydroxyl, lower alkoxy, lower alkyl, halogen, nitro, carboxyl, trifluoromethyl, amino, acyloxy, phenyl and naphthyl which are optionally substituted with halogen, nitro, lower alkoxy, and lower alkyl; or R is a monocyclic or bicyclic heterocyclyl radical in which 1 to about 3 heteroatoms are independently selected from nitrogen, oxygen or sulfur and said heterocyclyl radicals may be optionally substituted with one or more groups selected from alkyl having 1 to 6 carbon atoms, alkoxy having 1 to 6 carbon atoms, halo, and hydroxy;

W is selected from the group consisting of hydrogen, lower alkyl radicals, lower alkenyl radicals, lower alkynyl radicals, alicyclic hydrocarbon radicals and aromatic hydrocarbon radicals, wherein all of said radicals are optionally substituted with hydroxyl, lower alkyl, lower alkoxy, halog n, nitro, amino, acyloxy, phenyl

and naphthyl which may be optionally substituted with halogen, nitro, lower alkoxy, and lower alkyl;

Z, Z' are independently selected from the group consisting of hydrogen, lower alkyl radicals, halogen, alkoxy, cyano, sulfonyl, and hydroxy radicals;

m is an integer from 0 to about 6;

n is an integer from 0 to about 3;

n' is an integer from 0 to about 3.

2. A compound according to claim 1 wherein R is independently selected from the group consisting of hydrogen, lower alkyl radicals of 1 to about 6 carbon atoms, lower alkenyl radicals of 2 to about 6 carbon atoms, lower alkynyl radicals of 2 to about 8 carbon atoms, alicyclic hydrocarbon radicals of 3 to about 6 carbon atoms, and aromatic hydrocarbon radicals, wherein all of said radicals are optionally substituted with hydroxyl, lower alkoxy, lower alkyl, halogen, nitro, carboxyl, and trifluoromethyl; or R is a monocyclic or bicyclic heterocyclyl radical in which 1 to about 3 heteroatoms are independently selected from nitrogen or oxygen;

W is selected from the group consisting of hydrogen, lower alkyl radicals of 1 to about 6 carbon atoms, lower alkenyl radicals of 2 to about 6 carbon atoms, alicyclic hydrocarbon radicals of 3 to about 6 carbon atoms, and aromatic hydrocarbon radicals of 6 to about 12 carbon atoms, wherein all of said radicals are optionally substituted with hydroxyl, lower alkoxy, lower alkyl, halogen, nitro, amino, and acyloxy;

Z, Z' are independently selected from the group consisting of hydrogen, lower alkyl radicals, halogen, alkoxy, cyano, sulfonyl, and hydroxy radicals;

m is an integer from 0 to about 6;

n is an integer from 0 to about 3;

n' is an integer from 0 to about 3.

3. A compound according to claim 1 wherein R is independently selected from the group consisting of hydrogen, lower alkyl radicals and aromatic hydrocarbon radicals, or R is a monocyclic heterocyclyl radical in which 1 to about 2 hetero atoms are nitrogen;

W is selected from the group consisting of hydrogen and lower alkyl radicals;

Z, Z' are independently selected from the group consisting of hydrogen, lower alkyl radicals, halogen, and hydroxy radicals;

m is an integer from 0 to about 3;

n is an integer from 0 to about 1;

n' is an integer from 1 to about 2.

4. A compound according to claim 1 which is selected from the group consisting of:

(a) Ethyl β-[[N-3[[[4(aminoiminomethyl)phenyl]amino] carbonyl]piperidylcarbonyl]amino]-3-pyridinepropanoate,

(b) β-[[N-3-[[[4(aminoiminomethyl)phenyl]amino] carbonyl]piperidylcarbonyl]amino]-3-pyridinepropanoic acid,

(c) Ethyl β-[[N-2-[[[4(aminoiminomethyl)phenyl]amino] carbonyl]pyrrolidylcarbonyl]amino]-3-pyridinepropanoate,

(d) β-[[N-2-[[[4(aminoiminomethyl)phenyl]amino] carbonyl]pyrrolidylcarbonyl]amino]-3-pyridinepropanoic acid,

( ) Ethyl β-[[N-[[[4-(aminoimin methyl)ph nyl]amino]carbonyl]4-pip ridylcarbonyl]-amino]-3-pyridinepropanoate,

(f) β-[[N-[[[4-(aminoiminomethyl)phenyl]amino]carbonyl]4-piperidylcarbonyl]amino]-3-pyridinepropanoic acid.

5. A pharmaceutical composition comprising at least one non-toxic pharmaceutically acceptable carrier and at least one compound according to Claims 1, 2, 3 or 4 together with said carrier.

6. A method of treating a mammal to inhibit platelet aggregation comprising administering a therapeutically effective amount of at least one compound of Claims 1, 2, 3, or 4 to a mammal in need of such treatment.

INTERNATIONAL SEARCH REPORT

Inten ıal Application No
PCT/US 94/00513

A. CLASSIFICATION OF SUBJECT MATTER
IPC 5 C07D401/12 A61K31/44 //(C07D401/12,213:00,211:00), (C07D401/12,213:00,207:00)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 5 C07D A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP,A,0 513 810 (G.D. SEARLE & CO.) 19 November 1992 cited in the application see claim 1 ---	1-5
A	EP,A,0 445 796 (F.HOFFMANN-LA ROCHE AG) 11 September 1991 cited in the application see claim 1 -----	1-5

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

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Date of the actual completion of the international search	Date of mailing of the international search report
21 April 1994	16. 05. 94

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Gettins, M

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INTERNATIONAL SEARCH REPORT

Int. onal Application No
PCT/US 94/00513

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